QUANTIFICATION OF VALSARTAN BY UV-VISIBLE AND NMR SPECTROSCOPY

A dissertation submitted to

The Tamilnadu Dr. M.G.R. Medical University

Chennai – 600 032.

In partial fulfillment of the requirements
for the award of the degree of

MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by

Reg. No. 26108333

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
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Dr. A.JERAD SURESH, M.Pharm., Ph.D.

Principal,

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College of Pharmacy,

Madras Medical College ,

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CONTENTS

LIST OF ABBREVIATIONS

S.No	ABBREVIATION	EXPANSION
1	**VAL**	**Valsartan**
2.	Avg	Average
3.	LOD	Limit of Detection
4.	LOQ	Limit of Quantification
5.	NAOH	Sodium hydroxide
5.	mM	Millimoles
6.	MeOH	Methanol
7.	µg	Microgram
8.	µL	Microlitre
9.	R.S.D	Relative standard deviation
10.	R_f	Retention factor
11.	R_t	Retention time
12.	Std.	Standard
13.	S.D	Standard Deviation
14.	SE	Standard error
15	UV	Ultra-Violet Spectroscopy
16	Wt	Weight
17	QNMR	Quantization by Nuclear Magnetic Resonance Spectroscopy.

INTRODUCTION

Analytical chemistry, which is both a theoretical and practical science is practiced in large number of laboratories in diverse ways. Methods of analysis are routinely developed, modified, validated and applied for regular use. It is concerned with providing qualitative and quantitative information about the chemical and structural composition of a sample of matter. A million levels of drugs in biological tissue, are handled by the analyst. The field is huge variety of samples, from high concentrations of elements in alloy steels to part-per- founded on the conversion of a measured physical property of the species being examined to a usable signal. The overall strategy is to prepare a sample correctly, choose a particular method of analysis, and report the results in a meaningful format, which may include a statistical evaluation.

Analytical Chemistry deals with methods for determining the chemical composition of samples of matter. A qualitative method yields information about the identity and purity of atomic or molecular species or the functional groups in the sample; a quantitative method, in contrast, provides numerical information as to the relative amount of one or more of these components. Some of the recent analytical application includes:

- Determination of oxygen and CO_2 levels in millions of blood samples are carried out on a routine day to day basis, to diagnose and treat various ailments.

- Estimation of hydrocarbons, nitrogen acids and carbon monoxide present in automobile gases are done to assess the effectiveness of smog control devices.
- Analysis of calcium levels in blood serum help diagnose parathyroid disease.
- Protein content and nutritional value of food is established by estimating the nitrogen content.
- Steel with desired strength, hardness, corrosive resistance and ductility could be Produced by analyzing the concentration of elements such as carbon, nickel and Chromium and suitable manipulation.

Quantitative analysis plays a significant role in many research areas in chemistry, biology, geology, material science, engineering and medicine. The various technique of analytical chemistry is applied in all branches of chemistry (Skoog, *et al* 2004).

Analytical chemistry involves two basic divisions.

1. Qualitative analysis
2. Quantitative analysis

Qualitative analysis gives an indication of the identity of the chemical species in the sample and **quantitative analysis** determines the amount of one or more of these components. The separation of components is often performed prior to analysis.

Analytical methods can be separated into

- **Classical methods**
- **Instrumental methods.**

The separation of materials is accomplished using chromatography or electrophoresis methods. Analytical chemistry is also focused on improvements in experimental design, chemo metrics, and the creation of new measurement tools to provide better chemical information. Analytical chemistry has applications in forensics, bioanalysis, clinical analysis, environmental analysis, and material analysis.

Classical Methods

- Qualitative analysis
- Chemical tests
- Flame test
- Gravimetric analysis
- Volumetric analy**sis**

Instrumental methods

- Spectroscopy
- Mass spectrometry
- Electrochemical analysis
- Thermal analysis
- Separation
- Hybrid techniques
- Microscopy

Spectroscopy (http://en.wikipedia.org/wiki/Dual_polarisation_interferometry)

Spectroscopy measures the interaction of the molecules with electromagnetic radiation. It consists of many different applications such as atomic absorption spectroscopy, atomic emission spectroscopy, ultraviolet-visible spectroscopy, x-ray fluorescence spectroscopy, infrared spectroscopy, Raman spectroscopy, nuclear magnetic resonance spectroscopy, photoemission spectroscopy, Mossbauer spectroscopy and so on.

Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) spectroscopy is an analytical procedure based on the magnetic properties of certain atomic nuclei. It is similar to other types of spectroscopy in that absorption or emission of electromagnetic energy at characteristic frequencies provides analytical information.

NMR spectroscopy has been used for a wide range of applications such as structure elucidation; thermodynamic, kinetic, mechanistic studies, and quantitative analysis. Some of these applications are beyond the scope of compendia methods. (USP 2004-24). All five characteristics of the signal-chemical shift multiplicity, line width, coupling constants, and relative intensity contribute analytical information.

NMR spectrum is normally used for the following purpose:

- Identification of analyte
- Structure elucidiation of analyte
- To detect impurities
- To conduct degradation studies
- To investigate metabolic pathway

Comparison of spectrum from the literature or from an authentic specimen with that of a test specimen may be used to confirm the identity of a compound and to detect the **QNMR** presence of impurities that generate extraneous signals. The NMR spectra of simple structures can be adequately described by the numeric value of the chemical shifts and coupling constants and by the number of protons under each signal. Presently due to availability of high sensitivity/ homogeneity NMR instruments applications of qNMR has increased.

QNMR came into practice more than 5 decades ago and first paper on pharmaceutical was published in 1963 [D.P.Hollis., *et al.,* (1963)] NMR spectroscopy is a primary ratio method of measurement as the peak areas are proportional to the number of corresponding nuclei giving rise to signals. [H.Jancke.,*et al.,* (1998)] .The uncertainty in quantification measurement by NMR is low. [G. Maniara., *et al.,* (1998) The absolute amount of substances can be determined by using simple reference substances.

The advantages of qNMR are (Torgny Rundlof *et al.,* 2010) as follows:

- Simple and easy sample preparation
- Possibility to simultaneously determine molecular structure
- Individual experimental set up is not required.
- Relatively short measuring time
- Non invasive and nondestructive method
- Prior separation of analyte from mixture is not necessary
- Possibility of simultaneous quantification is of multiple target analyte in a mixture.

Quantitative analysis, as well as detection of trace impurities, is markedly improved with modern instrumentation. Stronger magnetic fields and the ability to accumulate and / or average signals over long periods of time greatly enhance the sensitivity of the method.

Quantitative Applications

If appropriate instrument settings for quantitative analysis have been made, the area (or intensities) of two signals are proportional to the total number of protons generating the signals

$$\frac{A_1}{A_2} = \frac{N_1}{N_2}$$

If the two signals originate from two functional groups of the same molecule, the equation can be simplified to

$$\frac{A_1}{A_2} = \frac{n_1}{n_2}$$

In which n1 and n2 are the number of protons in the respective functional groups. If the two signals originate from different molecular species.

$$\frac{A_1}{A_2} = \frac{n_1 m_1}{n_2 m_2} = \frac{n_1 W_1 / M_1}{n_2 W_2 m_2}$$

Where m1 and m_2 are the number of moles, W_1 and W_2 are the masses, and M1 and M_2 are the molecular weights of compounds I and 2 respectively.

The above equations indicated that NMR quantitative analysis can be performed in an absolute or relative manner. In the absolute method, an internal standard is added to the specimen and a resonance peak area from the internal standard. If both test substance and internal standard are accurately weighed, the absolute purity of the substance may be calculated. The relative method may be used to determine the molar fraction of an impurity in a test substance (or of the components in a mixture).

Hybrid techniques

Combinations of the above techniques produce a "hybrid" or "hyphenated" technique. Several examples are in popular use today and new hybrid techniques are under development. For example, gas chromatography-mass spectrometry, gas chromatography-infrared spectroscopy, liquid chromatography-mass spectrometry, liquid chromatography-NMR spectroscopy. Liquid chromagraphy-infrared spectroscopy and capillary electrophoresis-mass spectrometry.

A hyphenated separation technique refers to a combination of two (or more) techniques to detect and separate chemicals from solutions. Most often the other technique is some form of chromatography. Hyphenated techniques are widely used in chemistry and biochemistry.

Establishment of concentration of analyte

Standard curve

A general method for analysis of concentration involves the creation of a calibration curve. This allows for determination of the amount of a chemical in a material by comparing the results of unknown sample to those of a series known standards. If the concentration of element or compound in a sample is too high for the detection range of the technique, it can simply be diluted in a pure solvent. If the amount in the sample is below an instrument's range of measurement, the method of addition can be used. In this method a known quantity of the element or compound under study is added, and the

difference between the concentration added, and the concentration observed is the amount actually in the sample.

Internal standards

Sometimes an internal standard is added at a known concentration directly to an analytical sample to aid in quantitation. The amount of analyte present is then determined relative to the internal standard as a calibrate.

Standard addition

The method of standard addition is used in instrumental analysis to determine concentration of a substance (analyte) in an unknown sample by comparison to a set of samples of known concentration, similar to using a calibration curve. Standard addition can be applied to most analytical techniques and is used instead of a calibration curve to solve the matrix effect problem.

Statistical Parameters

Statistics consists of a set methods and rules for organizing and interpreting observations. The precision and reproducibility of the analytical method is determined by repeating the analysis. The following statistical parameters were calculated for the analytical methods employed.

Mean

The Mean or average is obtained by dividing the sum of observed values by the number of observations (n).

$$\bar{x} = \frac{\sum X}{n}$$

Standard Deviation

Standard deviation (SD) is measure of data dispersion or variability. The standard deviation gives an idea of how close the entire set of data is to the average value. SD is also called root mean square deviation.

$$SD = \sum (X - \bar{X})^2 / \sqrt{n-1}$$

Relative Standard Deviation

The relative standard deviation (RSD) is also called co-efficient of variation. It is defined as

$$RSD = SD / X$$

$$\%RSD = \frac{SD}{X} \times 100$$

Regression Equation

A regression is a statistical analysis assessing the association between two variables. It is used to find the relationship between two variables.

Regression equation $y = mx + c$

Where, m – the slope of regression line

c– the intercept point of regression line and the y axis

Standard Error

Standard error is a statistical term that measures the accuracy with which a sample represents a population. In statistics, a sample mean deviates from the actual mean of a population; this deviation is the standard error. Standard error (SE) is given by

$$SE = \frac{SD}{\sqrt{n}}$$

Validation

It is process which involves confirmation or establishment by laboratory studies that a method/procedure gives accurate and reproducible result for the intended analytical application in a proven and established range.

Types of validation (Sethi PD, 2008)

* Prospective validation

* Retrospective validation

* Concurrent

Prospective validation:

This is employed when historical data of the product is not available. Such validation is conducted prior to release of either new product or product made under revised/ new manufacturing process where revision may affect the product characters.

Retrospective validation:

This provides a review and evaluation of existing information for comparison when historical data is sufficient and readily available. Retrospective validation is acceptable provided specific test results generated by reliable analysis.

Concurrent:

Concurrent validation verifies the quality characteristics of a particular batch and provides assurance that the same quality would be attained again when subsequent batches are manufactured and analyzed under similar conditions.(P.D Sethi, 2008)

The types of analytical procedures used for validation are

* Identification tests.

* Quantitative tests for impurity content.

* Limit tests for the control of impurities.

* Quantitative tests of the active moiety in samples of substance.

Identification tests:

They are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample like spectrum, chromatographic behavior, chemical reactivity, etc. to that of a reference standard.

Test for impurities:

The testing for impurities can be a quantitative test or a limit test. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test.

Quantitative test:

Assay procedures are intended to measure the analyte present in a given sample. It represents a quantitative measurement of the major component(s) present in the drug.

Reasons for validation

* Enables scientists to communicate scientifically and effectively on technical matters
* Setting standards of evaluation procedure for checking complaints and taking remedial measures.

* Retrospective validation is useful for trend comparison of results compliance to CGMP/GLP.

* Closer interaction with pharmacopoeia harmonization particularly in respect to determination of impurities and their limits.

* For taking appropriate action, in case of non-compliance.

* Economic: The consistency and reliability of validated analytical procedure is to produce a quality product with all the quality attributes, thus providing indirect cost saving from reduced testing or retesting and elimination of product rejection.

* As quality control process is not static, some form of validation/ verification should continue till the validated process is in use.

Validation parameters (ICH Code Q2AC Guideline)

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated .The typical validation characteristics that are to be considered are listed below:

- Accuracy
- Precision
- Specificity
- Detection Limit
- Quantization Limit
- Linearity
- Range
- Robustness

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

Accuracy (Trueness)

The accuracy of an analytical procedure expresses the closeness of the test results obtained by that method to the true value (a conventional true value or an accepted reference)

Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample, or as the difference between mean and the accepted true value, together with confidence intervals.

The ICH documents recommend that accuracy be assessed using a minimum of nine determinations over a minimum of three concentration level covering the specified range (i.e., three concentrations and three replicates of each concentration)

Precision

The precision of an analytical procedure is the degree of agreement among the individual test result when the method is applied repeatedly the multiple samplings of a homogeneous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of a series of measurements. Precision may be a measure of either the degree of reproducibility or repeatability of the analytical method under operating conditions.

Repeatability:

It refers to the use of the analytical procedure within the laboratory over a short period of time using the same analyst with same equipment.

Reproducibility:

It refers to the use of analytical procedure in different laboratories as in a collaborative study.

Intermediate precision:

It expresses within laboratory variation, on the different days or different analysts or equipment within the same laboratory. ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure. (i.e., three concentrations) or a minimum of six determinations at 100% of the test concentration)

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Lack of specificity of an individual procedure may be compensated by other supporting analytical procedures.

Detection limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. Based on the standard deviation of the response and the slope, the detection limit of detection (LOD) may be expressed as **$DL=3.3\sigma/S$,** where σ is the standard deviation of the response and S is the slope of the calibration curve (of the analyte)

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. It is a parameter used particularly for the determination of impurities and/or degradation products. The quantization limit expressed as the concentration of analyte (e.g., percentage parts per million) in the sample.

$$LOQ = 10\sigma/S$$

Where σ = residual standard deviation of the response; S= slope of the calibration curve (of the analyte)

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amount) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, and linearity.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same sample under variety of conditions, such as different laboratories, analysts, instruments, lots of reagents, elapsed times, assay temperatures or days. Intermediate precision can be considered as ruggedness.

Robustness

The robustness of an analytical is a measure of its capacity to remain unaffected by small deliberate variations in methods parameters and provides an indication of its reliability during normal usage.

REVIEW OF LITERATURE

Eda Satana and Sadi Altany., *et al.,* (2001) designed the first-derivative Ultraviolet spectrophotometry and LC of simultaneous determination of Valsartan and hydrochlorothiazide in tablets.

Noriko Daneshtalab., et al., (2002) developed an assay method for the quantitation of the angiotensin II receptor antagonist valsartan in human plasma using a liquid extraction method. It is a convenient method which is suitable for pharmacokinetic studies.

Jingkai Gu., *et al.,* (2007) developed a liquid chromatography/tandem mass spectrometry method for the simultaneous quantification of valsartan and hydrochlorothiazide in human plasma which was successfully employed in a pharmacokinetic study.

Nozomu Kosek., *et al.,* (2007) carried out a method for the quantitative determination of valsartan in human plasma by liquid chromatography-tandem mass spectrometry (LC-MS)

Chitlange SS., *et al.,* (2008) described a precise, accurate and reproducible reverse phase High Performance Liquid chromatographic (RP-HPLC) method for estimation of amlodipine besylate and valsartan.

Oskar Gonzalez., *et al.,* (2009) developed SPE-HPLC-PDA- fluorescence method for the simultaneous determination of drugs used in combined cardiovascular therapy in human plasma.

Bhatia M. Sudesh., *et al.,* (2009) determined valsartan and its degradation products by isocratic HPLC.

K.R.Gupta., ***et al.,*** **(2010)** developed simultaneous estimation of valsartan and amlodipine and in their combined dosage form by UV spectrophotometric methods and validated the developed method.

Ch.Krishnaiah., ***et al.,*** **(2010)** developed stability-indicating gradient reverse phase ultra-performance liquid chromatographic (RP-UPLC) to determine their degradation products in active pharmaceutical ingredient and dosage form.

Ch Sharadha., ***et al.,*** **(2010)** developed validated RP-HPLC method for the quantitative estimation of valsartan in bulk and pharmaceutical dosage forms.

Chaudhary Ankit., ***et al.,*** **(2010)** estimated Valsartan & Nebivolol in pharmaceutical dosage forms by absorption ratio method.

Gaikwad AV., ***et al.,*** **(2011)** carried out the simultaneous estimation of ramipril and valsartan in capsules by HPTLC as per ICH guidelines.

Anandakumar K., ***et al.,*** **(2011)** designed an accurate and precise UV-spectrophotometric absorption correction method for simultaneous determination of amlodipine besylate, valsartan hydrochlorothiazide in bulk and in combined tablet dosage form.

Romina., ***et al.,*** **(2011)** determined the characterized two new potential impurities of valsartan obtained under photo degradation stress condition.

Bhaskara Raju V., ***et al.,*** **(2011)** performed reversed phase HPLC analysis of Valsartan in pharmaceutical dosage forms.

Della Grace Thomas Parambi., ***et al.,*** **(2011)** developed a validated stability indicating HPLC method for the determination of valsartan in tablet dosage forms.

DRUG PROFILE

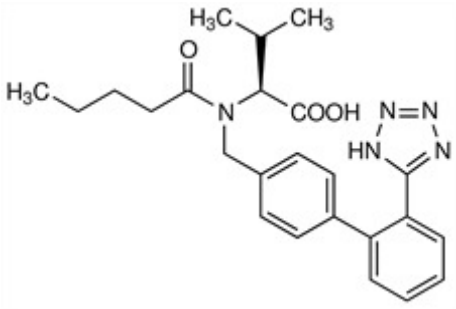
Valsartan

Valsartan is N-Pentanonyl- N-{2" (1H- tetrazol-5-yl) biphenyl-4-ylmethyl} –L-Valine

Empirical formula: $C_{24}H_{29}N_5O_3$

Molecular weight: 435.5g/mol

Structure:



Description

A white to almost white powder.

Melting point

116-117ºC

Solubility

The soluble in chloroform, and ethanol, methanol phosphate buffer pH4, 0.1 N Sodium hydroxide, and insoluble in 0.1 N Hydrochloric acid.

Mechanism of action

The final active messenger of the renin-angiotensin pathway is angiotensin II. Angiotensin II binds to Angiotensin1 receptors to cause vasoconstriction and fluid retention, both of which lead to an increase in blood pressure. The angiotensin II receptor blockers lower blood pressure by blocking the AT1 receptors. Therefore they have similar effects to angiotensin converting enzyme (ACE) inhibitors, which inhibit the synthesis of angiotensin II by ACE. However, non-ACE pathways can produce some angiotensin II. ACE inhibitors also decrease bradykinin breakdown and this action could be involved in some of the beneficial and adverse effects of that class of drugs. Therefore, a potential for differential clinical effects exists for these two classes of drugs.

Contra indications

Hypersensitivity; severe hepatic impairment, cirrhosis or biliary obstruction; primary hyperaldosteronism. Pregnancy (2nd and 3rd trimesters) and lactation.

Adverse drug reaction

Dizziness; headache; dose-related orthostatic hypotension; rash; angioedema; hyperkalaemia; myalgia; resp tract disorders; back pain; GI disturbances; fatigue; increase in BUN and serum creatinine; abdominal pain; dry cough; LFT elevations.

Potentially Fatal

Blood dyscrasias (e.g. neutropenia).

Drug interaction

Increased risk of renal impairment and hyperkalaemia with NSAIDs and ciclosporin. Increased risk of hypotension with general anaesthetics, clozapine, dopamine agonists and other antihypertensives. Increased risk of lithium toxicity. Increased risk of hyperkalaemia with potassium-sparing diuretics, potassium supplements, ACE inhibitors, and heparin.

Dosage forms

Only oral dosage form is available.

Dosage: Adult

Initially, 80 mg once daily, increased to 160 mg. Max: 320mg

Elderly

> 75 yr: Initially, 40 mg once daily.

Brands

Diovan, Valzaar

Precaution

Volume depletion; renal artery stenosis; monitor serum potassium concentrations; severe CHF; renal impairment; mild to moderate hepatic impairment, elderly.

AIM AND OBJECTIVE OF THE WORK

Standard analytical techniques are not available in the pharmacopoeias for newer drugs. So it becomes necessary to develop new analytical techniques for the newer drugs. Valsartan is one of the new drugs launched recently. The review of literature reveals that analytical techniques like HPLC, HPTLC, LC/MS have been reported for valsartan individually and in combination with other drugs but there is no evidence for the study of valsartan by UV using 0.1N sodium hydroxide, colorimetry involving acid dye method and quantitative NMR.

Thus the main objective of the work is to develop a simple, easy to perform, cost effective, accurate, precise and validated UV-Visible and qNMR methods for the estimation of Valsartan in bulk and tablet dosage forms. The methods developed include:

- **UV Spectroscopy**
  - Standard comparison method
  - Area Under the Curve
  - Derivative spectroscopy

  (First derivative, Second derivative)
  - Q-Absorbance Method
- **Visible Spectroscopy- Acid dye method**
  - Bromophenol and phosphate buffer pH 4

    Bromothymol blue and phosphate buffer pH 4
- **Q NMR – Quantization**

MATERIALS AND METHODS

Instrument employed

Shimadzu UV-Visible spectrophotometer, Model 1650 PC

Preparation of the standard stock solution

Aliquot quantity of standard valsartan was weighed in 100mL volumetric flasks. Dissolved in 10 ml of 0.1N sodium hydroxide and made up to volume with 0.1N sodium hydroxide. Dilutions ranging from 5-30µg/ml of standard valsartan solutions were prepared using 0.1N sodium hydroxide.

Preparation of the sample stock solution

The average weights of twenty tablets were accurately determined and powdered. Powdered sample equivalent to 100 mg of VAL was accurately weighed and transferred into a 100ml volumetric flask. The content was dissolved in 0.1N sodium hydroxide and shaken well. The solution was then allowed to stand for few minutes and made up to volume with 0.1N sodium hydroxide and filtered. The first few mL of the filtrate was discarded and the remaining solution was used for analysis.

ESTIMATION BY UV-SPECTROPHOTOMETRY (Metreyi 2008)

Most organic compounds absorb UV or visible light making them susceptible for quantification using spectrophotometers. Ultraviolet spectroscopy involves the measurement of light absorbed by the analyte present in the range of 200-350nm. The use of UV for quantitative analysis employs the method of comparing the absorbance of standards and samples at a selected wavelength

Criteria to select the procedure includes

- Conformity to Beer-Lamberts' law.
- Degree of selectivity and specificity.
- Stability of absorbance with respect to time, pH, ionic strength and temperature.

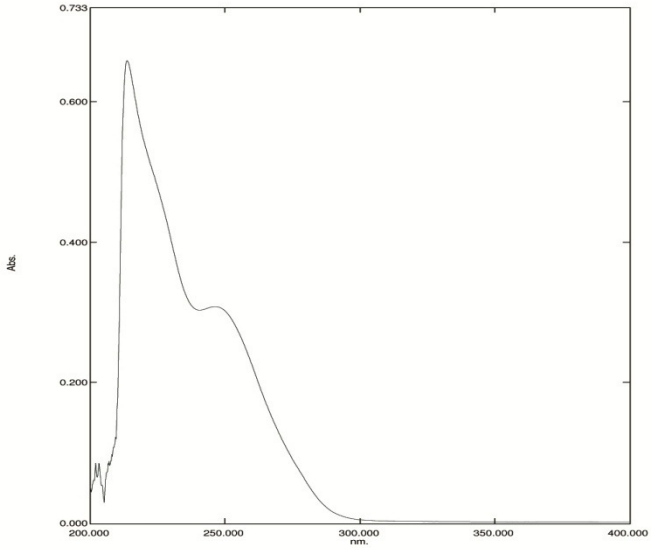
Establishment of various parameters

- ❖ Absorption maximum
- ❖ Beer's concentration
- ❖ Calibration graph
- ❖ Estimation of analyte in dosage form
- ❖ Recovery studies

Absorption Maximum

The standard stock solution was suitably diluted in 0.1N sodium hydroxide to yield a concentration of 10µg/mL. This solution was scanned in the UV region between 200-400nm using 0.1N sodium hydroxide as blank. It was found that VAL exhibited an intense maximum absorption at about 247nm.Fig-1

Fig-1 Absorption Spectra at 247nm



Selection of Solvent

The drug was found to be soluble in 0.1N sodium hydroxide.So, 0.1N sodium hydroxide was selected for the studies of valsartan by UV spectroscopy.

Beer's Concentration to confirm the linearity range

Aliquots of standard solution of VAL were suitably diluted to give varying concentrations ranging of 5-30µg/mL. The absorbances were measured at about 249nm and values are presented in **Table-1.**

Table 1: Absorbance of VAL at 249nm

Sl. No.	Concentration (in µg/mL)	Absorbance*
1	5	0.151
2	10	0.305
3	15	0.471
4	20	0.579
5	25	0.735
6	30	0.888

**Each value is the mean of three readings*

Calibration graph

A graph of absorbance against concentration was plotted. From the graph the Beer's law concentration for the analyte was found to be between 5-30µg/mL**(Fig-2).**

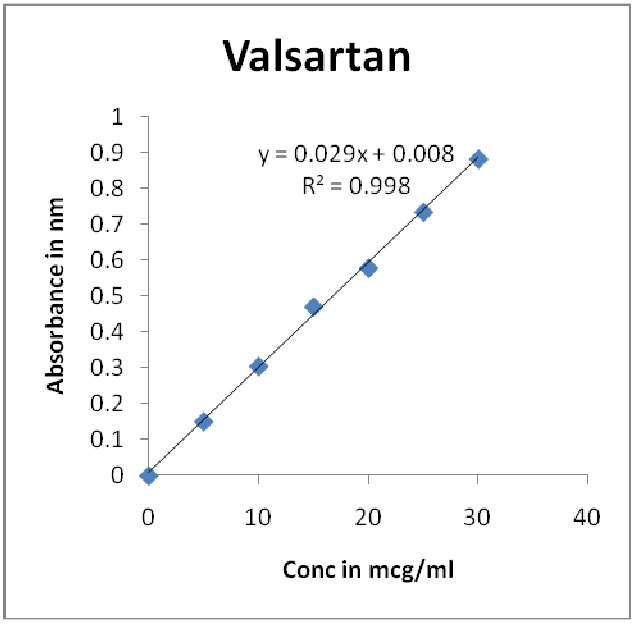
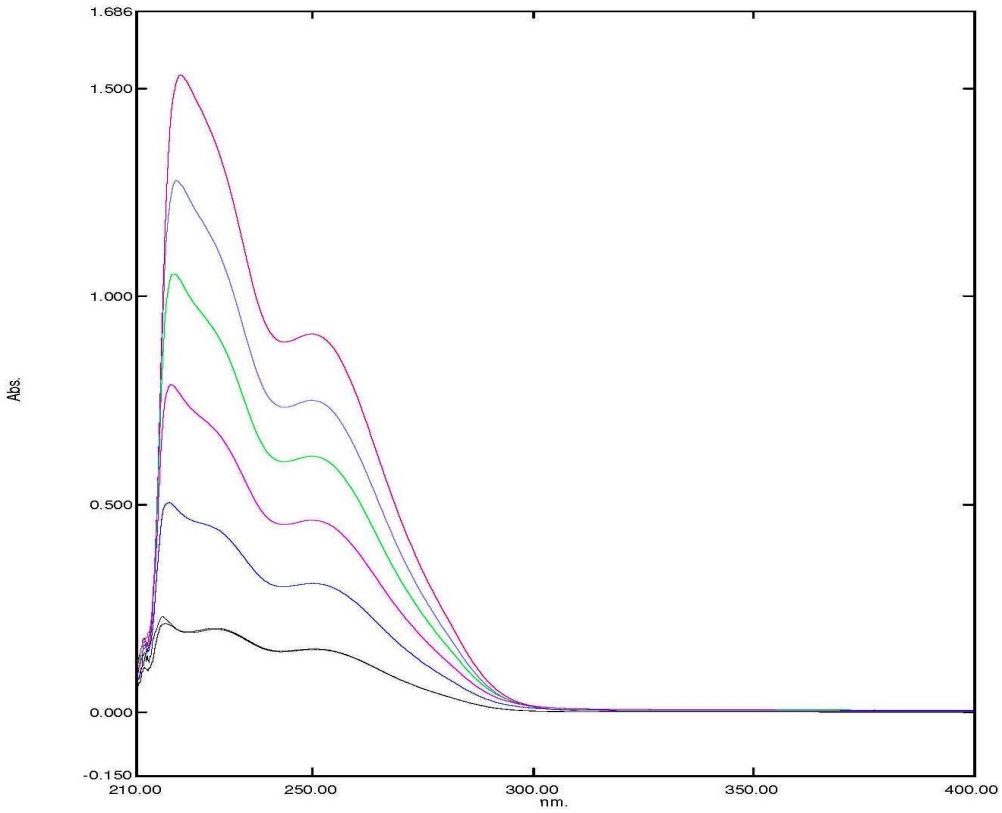


Fig 2: Calibration chart of VAL

Fig 3: Overlain spectra of VAL



Analysis of sample

The sample solution was further diluted with 0.1N sodium hydroxide and the absorbance of the solution was then measured at 249nm using 0.1N sodium hydroxide as blank. The amount of VAL was calculated using the formula

$$\textbf{Amount present} = \frac{\text{Sample absorbance}}{\text{Std absorbance}} \times \frac{\text{Dil. factor of standard}}{\text{Dil. factor of sample}} \times \frac{\text{Wt. of std.}}{\text{Wt. of sample}} \times \text{Avg. Wt}$$

Table 2: Results of Assay of VAL using Standard absorbance method

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	40mg	39.8 ± 0.00012	0.0031	99.48 ± 0.309	0.0031
2.		40.01 ± 0.00010	0.0026	100.25 ± 0.268	0.0026
3.		40.01 ± 0.00024	0.0061	100.36 ± 0.619	0.0061

**Each value is the mean of three determinations*

Recovery studies

To study the accuracy, precision and reproducibility of the proposed method, recovery studies were carried out by adding a known quantity of drug to pre-analyzed sample and the percentage recovery was calculated and the results obtained are presented in **Table-3.**

Table 3: Results of Recovery Studies of VAL using Standard Absorbance method

Expected % Recovery	Amount of Drug Added in mg		Total Amount Assayed (mg) *	Amount Rcovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	40mg	8	47.81	8.11	20.26	101.34 ± 0.154	0.0076
50%		20	59.78	20.07	50.17	100.35 ± 0.309	0.0061
100%		40	80.07	40.03	100.90	100.90 ± 0.268	0.002

Each value is the mean of three determinations.

METHOD 2: AREA UNDER CURVE METHOD (Niraimathi V, *et al.,* (2010)

This method is applicable when there is no peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ1 and λ2. The inbuilt software calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration (Mukesh, 2007).

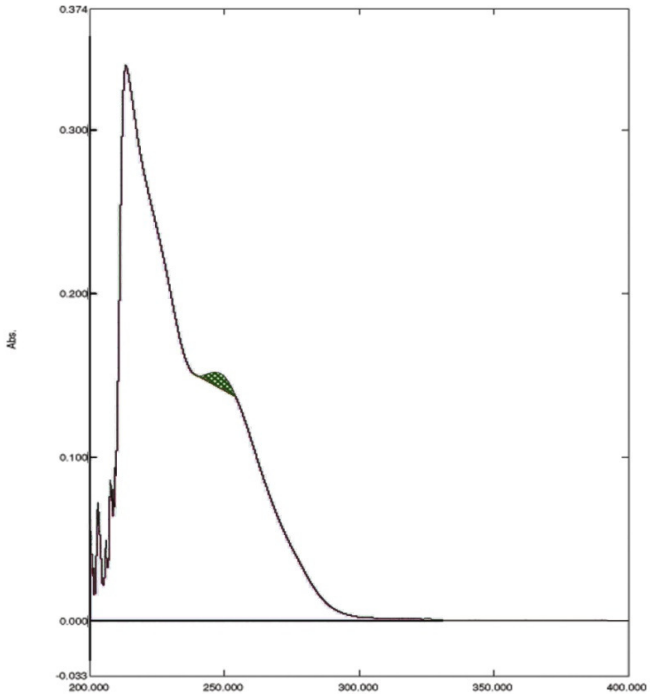
Establishment of various parameters

- Area under the curve
- Linearity
- Calibration graph
- Estimation of analyte in dosage from
- Recovery studies

Area under curve

The standard stock solution was suitably diluted in 0.1N sodium hydroxide to yield a concentrations ranging from 5-30µg/mL. This solution was scanned in the UV region between 200-350nm using 0.1N sodium hydroxide as blank. The calibration graph was plotted against AUC and the linearity was established. The sample AUC was interpolated on the respective linearity chart of the AUC and the concentration was determined. The two selected wavelength chosen for AUC determined was between 238-254nm.

Fig 4: AUC of VAL between 238-254nm.



Linearity range

The AUC obtained for different concentrations of standard solution of VAL are presented in **Table4.**

Table -4: AUC of VAL between 238-254

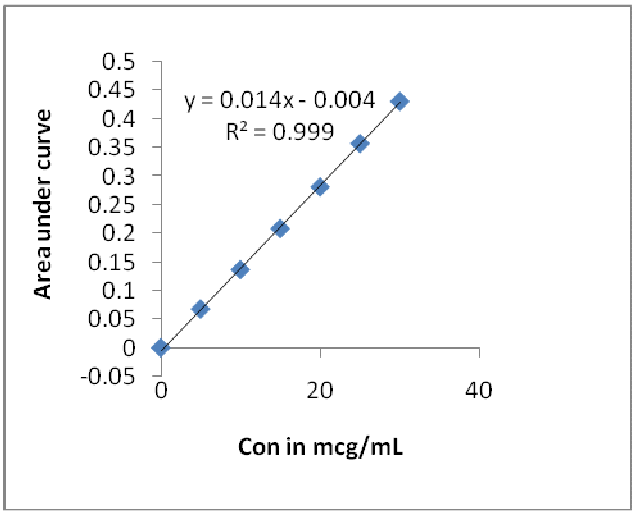
Sl. No.	Concentration (in µg/mL)	Absorbance*
1	5	0.068
2	10	0.137
3	15	0.209
4	20	0.282
5	25	0.358
6	30	0.432

*****Each value is the mean of three determinations*

Calibration graph

A graph of AUC against concentration was plotted. From the graph, it was found that the drug obeyed linearity in the range 5-30µg/mL (**Fig-5.**)

Fig 5: Calibration graph of VAL by AUC method



Analysis of sample

The sample solution was suitably diluted and was scanned in the spectrum mode and AUC was calculated in the wavelength range 238-254 nm. The AUC so obtained was interpolated on the calibration graph and the concentration of sample determined. The amount present per tablet was calculated and presented in **Table-5.**

Table 5: Results of Assay of VAL using AUC

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	40mg	40.04 ± 0.0004	0.0094	100.91 ± 0.9451	0.0094
2.		39.60 ± 0.0004	0.0096	98.91 ± 0.9451	0.0096
3.		40.01 ± 0.00070	0.0163	100.24 ± 1.6370	0.0163

**Each value is the mean of three determinations*

Recovery studies

To study the accuracy, precision and reproducibility of the proposed method, the recovery studies were carried out on spiked samples by adding predetermined amount of standard drugs to the respective sample. About 20, 40 and 100% of standard drugs were added to the sample solutions and the absorbance was measured against methanol blank. The percentage recovered was calculated and presented in **Table -6.**

Table 6: Results of Recovery Studies of VAL using AUC

Expected % Recovery	Amount of Drug Added in mg		Total Amount Assayed (mg) *	Amount Rcovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	40mg	8	48.9	8.05	20.11	100.58 ± 0.094	0.0047
50%		20	60.08	19.90	49.78	99.58 ± 1.25	0.0251
100%		40	80.10	40.10	100.18	100.18 ± 0.094	0.0009

*****_Each value is the mean of three determinations._

METHOD 3: FIRST DERIVATIVE SPECTROSCOPY (Chatwal 2005)

Derivative spectrophotometry involves the transformation of absorption spectra into first, second or higher order derivative spectra. In derivative spectroscopy, the ability to detect and to measure minor spectral features is considerably enhanced. It can be used in quantitative analysis to measure the concentration of an analyte whose peak is obscured by a larger overlapping peak. It is useful in eliminating matrix interference in the assay of many medicinal substances. Derivative spectrum is done by wavelength modulation with dual wavelength photometers and microprocessor controlled digital photometer.

Normal spectrum is a zero order spectrum. The first derivative (D1) spectrum is a plot of the gradient of absorption curve (rate of change of absorbance with wavelength ie. $dA/d\lambda$ Vs λ) against wavelength. It is characterized by a maximum, minimum and a cross over point at the λ_{max} of the absorption band

Advantages:

Accurate determination of λ max is possible.

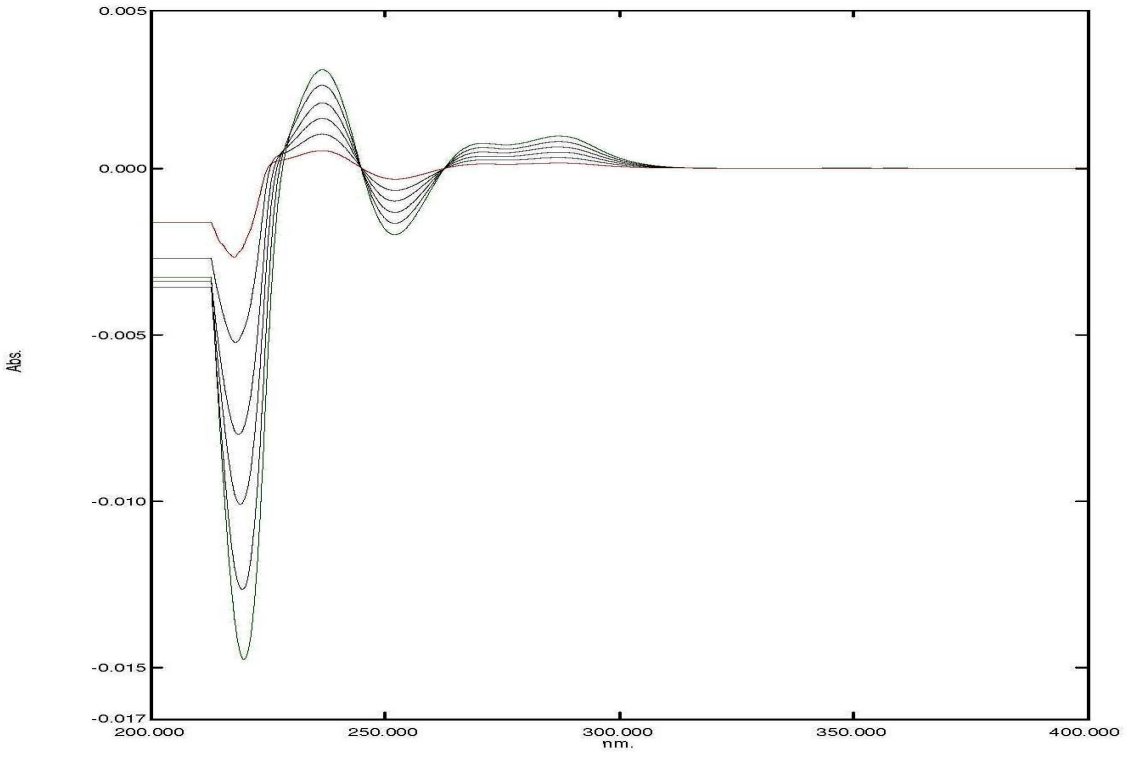
1. Increased resolution permits the selective determination of certain absorbing substances.
2. Absorption bands can be recognized when there are two or more absorption bands overlapping at the same or slightly different wavelength.

For quantification, peak heights (in mm) are usually measured. The amplitude is the distance from the maximum to the minimum at the λ max (which is the zero crossing in the spectrum) in first order. The amplitude of positive or negative peaks at the cross over point is directly proportional to the concentration.

Establishment of optimum parameters

The standard stock solution of VAL was suitably diluted to give the various concentrations ranging from 5-30µg/mL .These solutions were scanned between 200-400nm and the primary absorption spectra were recorded. The primary spectrum was then derivatized for the first order **(Fig 6)**

Fig -6: Overlain spectra of first derivative of Valsartan



The primary spectrum was then derivatized to the first order using derivative mode. The amplitude of the negative peak maximum at the zero crossing of the first order curve was measured in mm at 247nm and is presented in Table-7.

Table 7: Valsartan in mm for first derivative spectrum

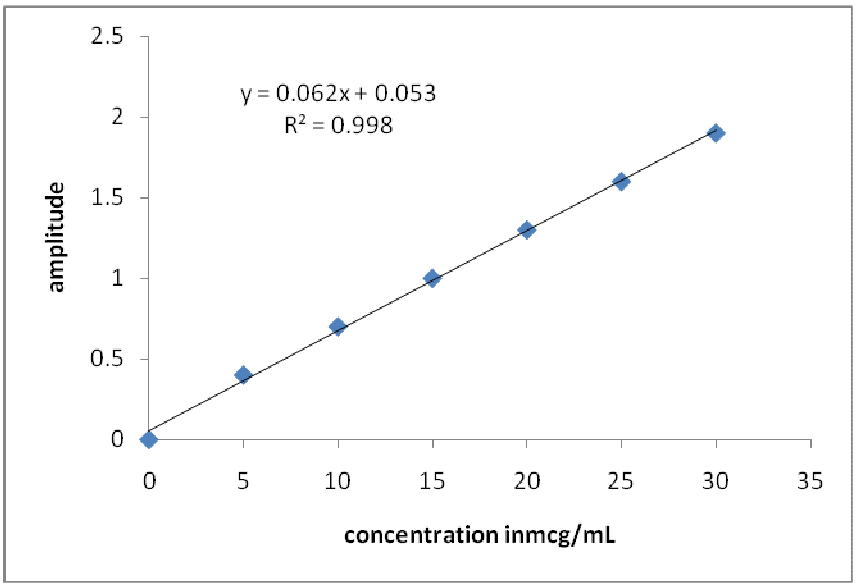
Sl. No.	Concentration (in µg/mL)	First Derviative Amplitude (mm)*
1	5	0.4
2	10	0.7
3	15	1.0
4	20	1.3
5	25	1.6
6	30	1.9

*****_Each value is the mean of three determinations._

Calibration graph

A graph was constructed by plotting amplitude against concentration and is shown in Fig.7 for first order. The Beer's Law was obeyed in the range of 5-30µg/mL for first derivative spectroscopic method.

Fig 7: Calibration graph of VAL by first derivative spectroscopy



Analysis of sample solution

The sample solution was suitably diluted and scanned between 200-400nm using 0.1N sodium hydroxide as blank and the primary spectrum obtained was derivatized for first order derivative. The amplitude (DL) of the peak maximum and minimum at the zero crossing of the first order curve (i.e. λmax of the fundamental spectrum) was measured in mm. The amount of drug present was found by interpolation on the calibration graph and the amount of drug present per tablet was calculated by using the formula and the assay results are given in the **Table 8**

Amount present $= \frac{\text{Sample absorbance}}{\text{Std absorbance}} X \frac{\text{Dil.factor of standard}}{\text{Dil.factor of sample}} X \frac{\text{Wt. of std.}}{\text{Wt. of sample}} X \text{Avg. Wt}$

Table 8: Results of Assay and Precision of VAL

(First derivative spectroscopy)

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	40mg	39.8 ± 0.0008	0.0190	98.24 ± 1.8903	0.0190
2.		40.1 ± 0.0005	0.0123	100.44 ± 1.2349	0.0123
3.		40.1 ± 0.0007	0.0163	100.24 ± 1.6370	0.0163

**Each value is the mean of three determinations*

Recovery Studies

To study the accuracy, precision and reproducibility of the proposed method, recovery study was carried out by adding a known quantity of drug to preanalysed sample and the percentage recovery was calculated and the results obtained are presented in **Table-9**

Table 9: Results of Recovery Studies of VAL

(First derivative spectroscopy)

Expected % Recovery	Amount of Drug Added in mg		Total Amount Assayed (mg) *	Amount Rcovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	40mg	8	48.01	8.10	20.18	100.90 ± 0.00004	0.0047
50%		20	60.18	20.24	50.59	101.18 ± 0.00004	0.0019
100%		40	79.58	39.60	99.11	99.11 ± 0.0001	0.0034

Each value is the mean of three determinations.

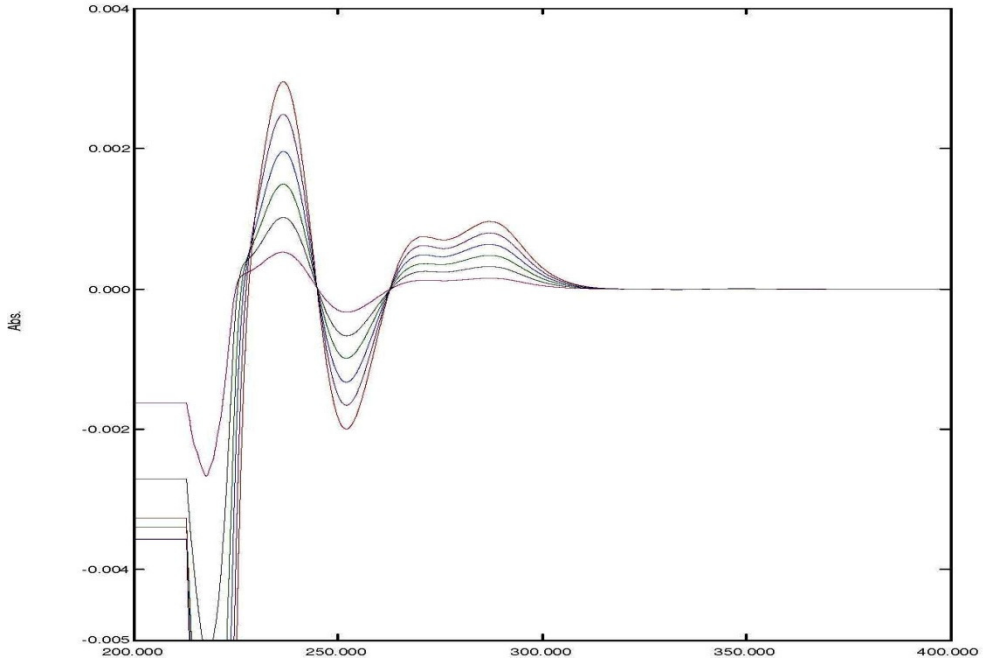
METHOD 4: SECOND DERIVATIVE SPECTROSCOPY (Beckett,1997)

Normal spectrum is a zero order spectrum. The primary spectrum obtained for the above was then derivatized for the second order. The second derivative spectrum (D2) is a plot of curvature of the absorption spectrum against wavelength (d^2 A / $d\lambda^2$ Vs λ). The amplitude (D_L) of long wave peak satellite of the second order curve was measured. The amplitude of the negative peak maximum corresponding to λmax of fundamental spectrum was measured.

Establishment of optimum parameters

The standard stock solution was suitably diluted to give the various concentrations ranging from 5-30µg/mL. These solutions were scanned between 200-400nm and the primary absorption spectra were recorded. The primary spectrum was then derivatized to the second order (Fig. 8)

Fig 8: Calibration graph of VAL by second derivative spectroscopy



The primary spectrum obtained above was then derivatized to the second order. The amplitude of the negative peak maximum was measured in mm at 247nm and is presented in **Table-10**

Table 10: Ampitude in mm for second derivative spectrum

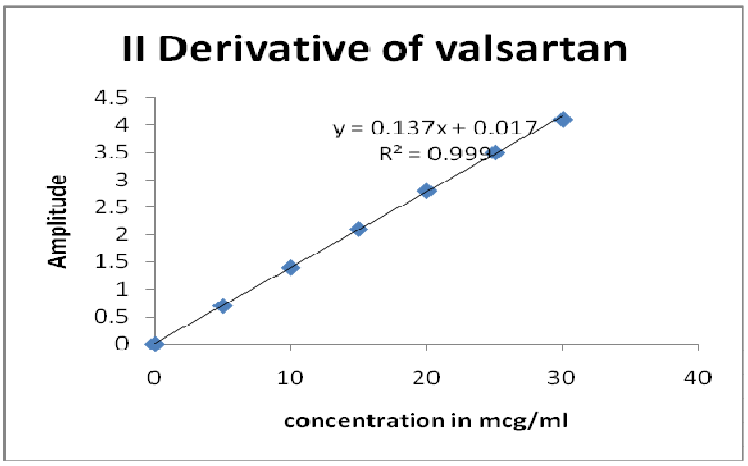
Sl. No.	Concentration (in µg/mL)	First Derviative Amplitude (mm)*
1	5	7
2	10	14
3	15	21
4	20	28
5	25	35
6	30	41

*****Each value is the mean of three determinations.*

Calibration graph

A graph was constructed by plotting amplitude against concentration and is shown in Fig. 9 for the second order. The Beer's Law was obeyed in the range of 5-30µg/mL for second derivative spectroscopic method.

Fig 9 Calibration graph of VAL by second derivative spectroscopy



Analysis of sample solution

The sample solution was suitably diluted and scanned between 200-400nm using 0.1N sodium hydroxide as blank and the primary spectrum obtained was derivatized for second order derivative. The amplitude (DL) of long wave peak satellite of the second order curve (i.e. λmax of the fundamental spectrum) was measured in mm. The amount of drug present was found by interpolation on the calibration graph and the amount present per tablet was calculated.The assay results are presented in the **Table 11**

Table 11: Results of Assay and Precision of VAL

(Second derivative spectroscopy)

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	40mg	39.8 ± 0.00012	0.0031	99.48 ± 0.309	0.0031
2.		40.01 ± 0.00010	0.0026	100.25 ± 0.268	0.0026
3.		40.01 ± 0.00024	0.0061	100.36 ± 0.619	0.0061

**Each value is the mean of three determinations*

Recovery studies

To study the accuracy, precision and reproducibility of the proposed method, recovery study was carried out by adding a known quantity of drug to preanalysed sample and the percentage recovery was calculated the results obtained are presented in **Table 12**

Table12: Results of Recovery Studies of VAL

(Second derivative spectroscopy)

Expected % Recovery	Amount of Drug Added in mg		Total Amount Assayed (mg) *	Amount Rcovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	40mg	8	48.9	8.05	20.11	100.58 ± 0.094	0.0047
50%		20	60.08	19.90	49.78	99.58 ± 1.25	0.0251
100%		40	80.10	40.10	100.18	100.18 ± 0.094	0.0009

*****_Each value is the mean of three determinations._

METHOD 5: Q- ABSORBANCE METHOD (Priyadarshini J, *et al.,* 2010)

Q- absorbance method depends on the property that, for a substance which obeys Beer's Law at all wavelength, the ratio of absorbances at two wavelengths is a constant value independent of concentration or pathlength (Beckett, 1997). The wavelengths selected for this method are 235 nm to 250nm. The ratio of absorbances between these two wavelengths was calculated. The values obtained by the proposed method are presented in **Table-13**

Table 13: Ratio of absorbances at 235 nm and 250 nm

S.No	Concentration (µg/mL)	Absorbance at 235nm	Absorbance at 250nm	Ratio of absorbance*
1	5	0.161	0.150	1.07
2	10	0.325	0.302	1.07
3	15	0.502	0.466	1.07
4	20	0.615	0.574	1.07
5	25	0.782	0.727	1.07
6	30	0.937	0.874	1.07

Establishment of optimum parameters

The standard stock solution of VAL was suitably diluted to give the various concentrations ranging from 5-30µg/mL. These solutions were scanned between 200-400nm and the primary absorption spectra were recorded. It was found that VAL exhibited an intense maximum absorption at about 235nm . The two wavelengths chosen for this method are 235-250nm respectively.

Analysis of Sample solution

The sample solution was suitably diluted and scanned between 200-400nm using 0.1N sodium hydroxide as blank and the absorbance of the solution was then measured at 235nm and 250nm using 0.1N sodium hydroxide as blank. The ratio of absorbances between these two wavelengths was calculated. The results are tabulated in **Table 14**

Table14: Results of Assay and Precision of VAL

(Q Absorbance)

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	40mg	39.9 ± 0.00010	0.0027	99.76 ± 0.2739	0.0027
2.		39.9 ± 0.00017	0.0043	99.98 ± 0.4320	0.0043
3.		39.80 ± 0.00011	0.0027	99.57 ± 0.2739	0.0027

**Each value is the mean of three determinations*

VISIBLE SPECTROPHOTOMETRY METHOD (COLORIMETRY)

Colorimetry (Vogel, 1989) involves the measurement of amount of visible radiation absorbed by substance in the solution. The wavelength between 400-800nm is considered to be visible region. The use of visible spectroscopy for quantitative analysis employs the method of comparing the absorbance of reference standards and samples at a selected wavelength. Various colorimetric methods include oxidation or reduction, diazotization followed by coupling, complexing with metal ion pair complexes with indicator dyes etc.

Advantages of colorimetric procedures

- A colorimetric method will often give more accurate results at low concentrations than the corresponding titrimetric or gravimetric procedure.
- It may be simpler to carry out.
- It may be frequently applied under conditions where no satisfactory gravimetric or titrimetric procedure exists.
- It possesses advantages over the routine determination of some components of a number of similar samples may be made

The criteria for the selection of procedure include:

- Specificity of the colour reaction
- Proportionality between colour and concentration
- Stability of the colour
- Reproducibility
- Clarity of the solution

- High sensitivity
- Stability of absorbance with respect to time, variation of pH,ionic strength and temperature.
- Degree of selectivity of complexing agent includes the effect of other species likely to be present
- Conformity to the Beer-Lambert's law and plot calibration data for the range of concentration measured.

METHOD 6: CHARGE TRANSFER COMPLEX METHOD

(Acid- Dye Method) Higuchi 1997

This method is based on the formation of colored ion-pair complex on utilization of acidic dyes as analytical reagents. The ion-pair complex is special form of molecular complex resulting from two components i.e the ionized analyte and an acid dye at suitable (pH 4).

The addition of an amine in its ionised form to an ionized acidic dye, e.g. bromocresol green, bromothymol blue, yields a salt (ion-pair).The indicator dye is added in excess and the pH of the aqueous solution is adjusted if necessary to a value where both the analyte and dye are in the ionised forms.The molar absorptivities of the ion-pairs formed between quaternary ammonium compounds and bromothymol blue are typically 2 x 10^4 -4 x 10^4. The acid-dye method therefore provides a more sensitive technique for certain analyte and quaternary ammonium compounds that absorb weakly in the ultraviolet region. The correct choice of pH may permit the selective assay of a mixture of an analyte and a quaternary ammonium salt.

Reagents and chemicals

0.1N sodium hydroxide as per I.P

Bromophenol blue solution (0.1%) as per I.P 1996 (P.A-202)

Phosphate buffer pH 4 as per I.P 1996 (P.A-146)

Method A: Bromophenol blue and phosphate buffer pH 4

Preparation of reagents

Bromophenol blue solution (0.1%) I.P 1996 (P.A-202)

Ethanolic Bromophenol blue Solution: Dissolve 0.1g of bromophenol blue with gentle heating in 1.5mL of 0.1M Sodium hydroxide and 20mL of ethanol (95%) and add sufficient water to produce 100mL.

Phosphate buffer pH 4 (IP, 1996 P.A-146)

Dissolved 5.04 gm of disodium hydrogen phosphate and 3.01 gm of potassium dihydrogen phosphate in sufficient water to produce 1000mL, pH adjusted with glacial acetic acid.

Establishment of optimum levels of various parameters

Absorption maximum

Stability of color

Beer's concentration

Calibration graph

Estimation of analyte in dosage form

Recovery studies

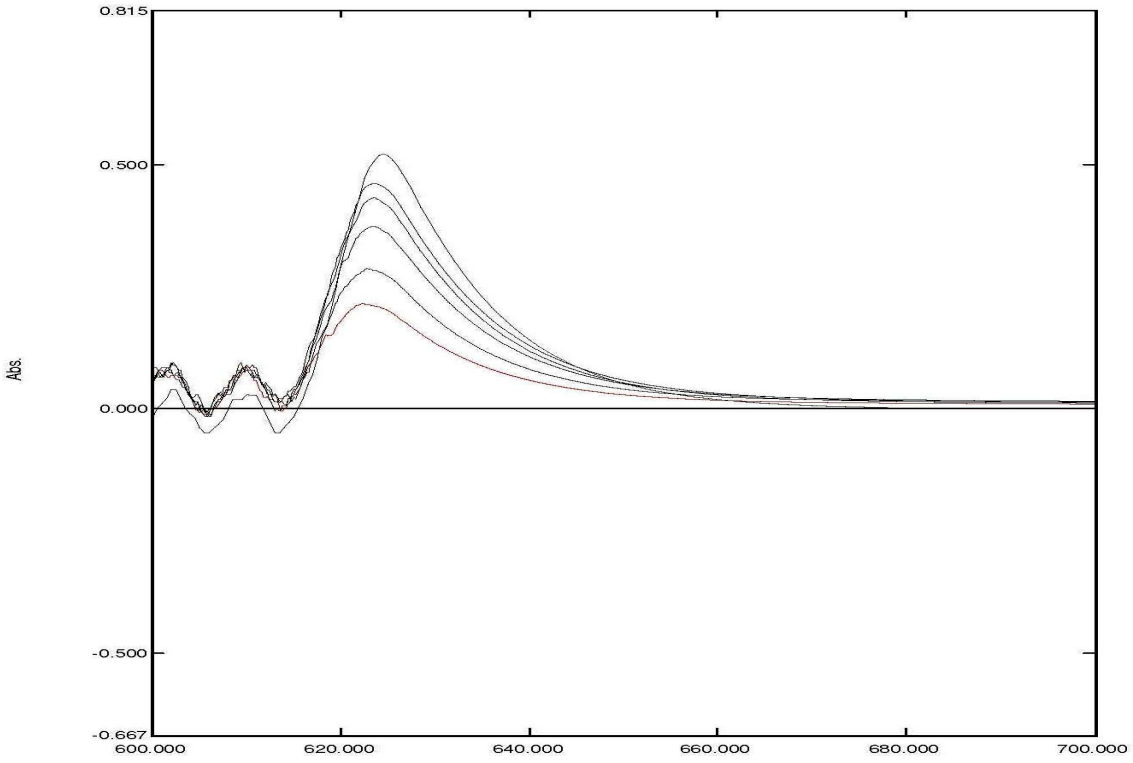
Stock solution (1mg /mL)

50mg of pure valsartan was accurately weighed and transferred into 50mL volumetric standard flask and to that phosphate buffer was dissolved to dissolve the drug and made up to 50mL with phosphate buffer.

Determination of Absorption Maximum (λmax)

The stock solution was suitably diluted to get a concentration of 1000µg/mL. Further dilutions containing 1mL, 2mL, 3mL, 4mL and 5mL of stock solution were transferred in to six 10 mL volumetric flasks. To this was added 1mL of 0.1% Bromophenol blue solution and the volume made upto 10mL with phosphate buffer. Chromogen was determined by scanning in the visible region when yellow colouration changes to blue violet and the absorption maximum was found to be 622nm. The spectrum is shown is in **Fig 10**

Fig 10: Absorption maximum of ion-pair complex at 622nm



Stability of colour

The absorbance of yellow to violet colour complex was measured at 622nm at 30min, 1 hr and 2hr against reagent blank. The colour was stable for 3hrs.

Evaluation of Beer's Concentration

Aliquots of the standard stock solution were transferred to a series of 10mL volumetric flask, 1mL of 0.1% Bromophenol solutions were added to the respective flasks. The volumes were then made up using in phosphate buffer pH4. The absorbance of the solution was measured at 622nm using reagent blank.

The absorbances obtained are presented in **Table 15**

Table 15 : Absorbance VAL - at 622nm

S.No	Concentration (in µg/mL)	Absorbance*
1	100	0.216
2	200	0.287
3	300	0.352
4	400	0.409
5	500	0.461
6	600	0.522

**Each value is the mean of three determination*

A buffer of pH 4 and a absorption maximum of 622nm was used for further studies

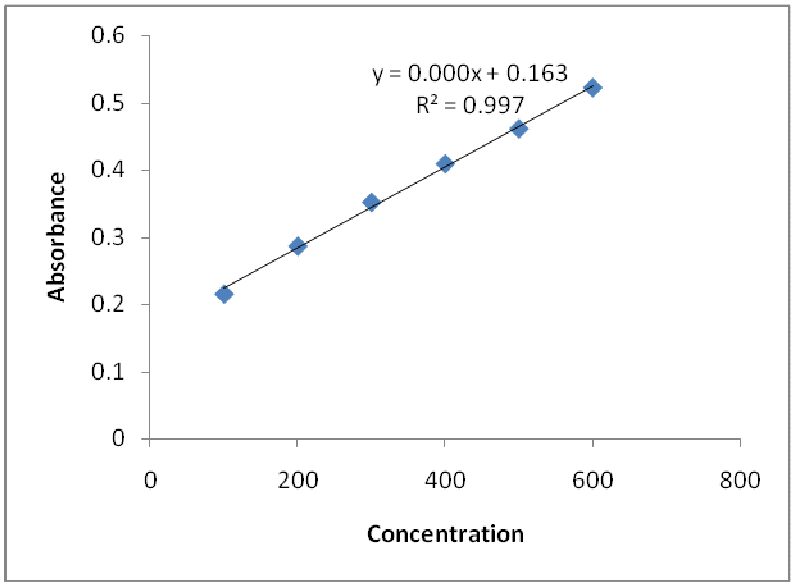
Beer's concentration

According to Beer's law, the graph was drawn by measuring the absorbance of various concentration of drug solution against the blank from stock solution (1µg/mL) of various concentrations 0.1mL, 0.2mL and 0.5mL were pipette out and transferred into 25mL volumetric flask and make up with phosphate buffer. The drug concentration in each flask was 5µ/mL,

Calibration graph

A graph was constructed by plotting the absorbance against concentration and is shown in **Fig11.** It was observed that VAL obeyed Beer's law in the concentration range of 100-600µg/mL.

Fig11: Calibration graph of Bromophenol Blue



Analysis of Sample solution:

Twenty tablets of VAL from the tablets were accurately and weighed and grand to fine powder. Tablet powder equivalent to 50mg of VAL was accurately weighed and shaken well with phosphate buffer for 20 minutes, and made up to volume with phosphate buffer to obtain a concentration of 1000µg/mL. The solution was filtered through Whatmann filter paper No.41.First 10 mL of the filtrate was discarded, and then 1mL of the filtrate was transferred to a 10mL volumetric flask. The sample solution was treated with acid dye and the absorbance of the chromogen was measured at the maximum at about 622nm using reagent blank.

$$\text{Amount Present} = \frac{\text{Sample absorbance}}{\text{Std absorbance}} \times \frac{\text{Dil. factor of standard}}{\text{Dil. factor of sample}} \times \frac{\text{Wt. of std.}}{\text{Wt. of sample}} \times \text{Avg. Wt}$$

Table16 : Results of Assay and Precision of VAL

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	40mg	40.32 ± 0.00005	0.0013	100.80 ± 0.1347	0.0013
2.		40.39 ± 0.00010	0.0026	100.99 ± 0.2694	0.0026
3.		40.47 ± 0.00016	0.0039	101.18 ± 0.4042	0.0039

**Each value is the mean of three determinations*

Recovery studies

To study the accuracy, precision and reproducibility of the proposed method, recovery study was carried out by adding a known quantity of drug to preanalysed sample and the percentage recovery was calculated and the results obtained are presented in **Table 17**

Table 17: Results of Recovery Studies of VAL

Expected % Recovery	Amount of Drug Added in mg		Total Amount Assayed (mg) *	Amount Rcovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	40mg	8	48.36	8.06	20.15	100.76 ± 0.233	0.0115
50%		20	60.41	20.10	50.26	100.52±0.1347	0.0028
100%		40	80.68	40.40	100.95	100.95 ± 0.1347	0.0013

*****_Each value is the mean of three determinations._

METHOD B: **Acid dye using Bromothymol blue** (L.Latheeshjlal

Reagents and chemicals

0.1N sodium hydroxide

Bromothymol blue solution (0.1%)

Phosphate buffer pH 4 as per I.P 1996 (p.A-146)

Preparation of reagents

Bromothymol Blue Solution:

Aqueous Bromothymol Blue Solution. Dissolve 50mg of bromothymol blue in 4mL of 0.02M sodium hydroxide and 20mL of ethanol (95%).After solution is effected, add sufficient water to produce 100mL.

Phosphate buffer pH 4

Dissolved 5.04 gm of disodium hydrogen phosphate and 3.01 gm of potassium dihydrogen phosphate in sufficient water to produce 1000mL, pH adjusted with glacial acetic acid

Stock solution (1mg /mL)

50mg of pure valsartan was accurately weighed and transferred into 50mL volumetric standard flask and dissolved in 0.1N sodium hydroxide. Finally made up to 50mL with phosphate buffer

Spectral characterization and Linearity Establishment

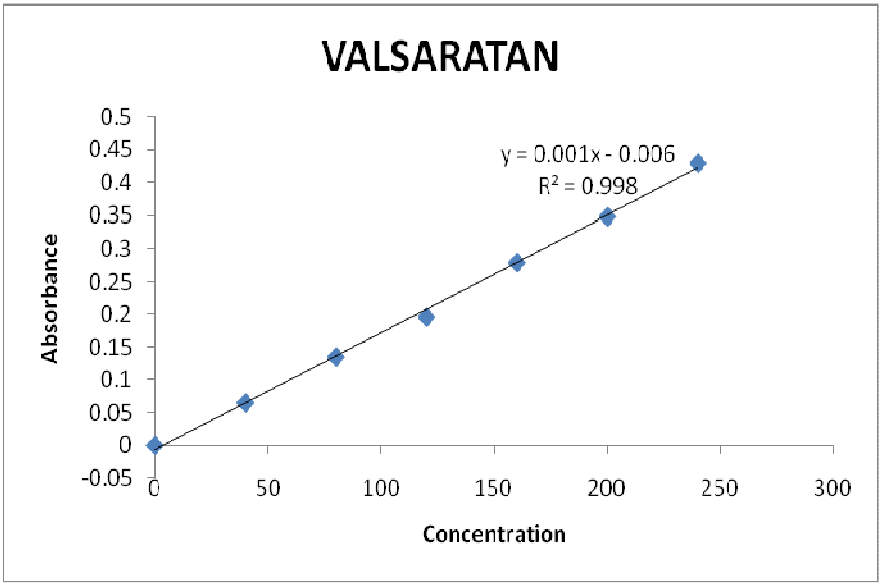
A stock solution of VAL was prepared by dissolving accurately weighed quantity of standard VAL in phosphate buffer pH 4 and made up to volume with phosphate buffer pH 4 to obtain a concentration of 1000µg/mL. From the stock solution 1.0, 2.0, 3.0, 4.0 and 5.0 were transferred into five 25 mL volumetric flasks. The solutions were added a 5mL of 0.1% of bromothymol blue solution and made upto 25mL with phosphate buffer, when red coloured changed in yellow colour.

Table18 : Absorbance VAL - at 433nm

S.No	Concentration (in µg/mL)	Absorbance*
1	40	0.065
2	80	0.135
3	120	0.196
4	160	0.279
5	200	0.348
6	240	0.429

**Each value is the mean of three determination*

Fig 12: Calibration graph of Bromothymol Blue



Sample solution:

Twenty tablets of VAL from the tablets were accurately and weighed and grand to fine powder. Tablet powder equivalent to 50mg of VAL was accurately weighed and dissolved in 1Msodium hydroxide and finally volume is made up with phosphate buffer to obtain a concentration of 1000µg/mL. The solution was filtered through Whatmann filter paper No.41. First 10 mL of the filtrate was discarded, and then 3mL of the filtrate was transferred to a 25 mL volumetric flask. The sample solution was acidic dye complexed and the absorbance was measured using the same procedure as that of standard VAL.

Fig 13 :Absorption maximum of ion-pair complex at 433nm.

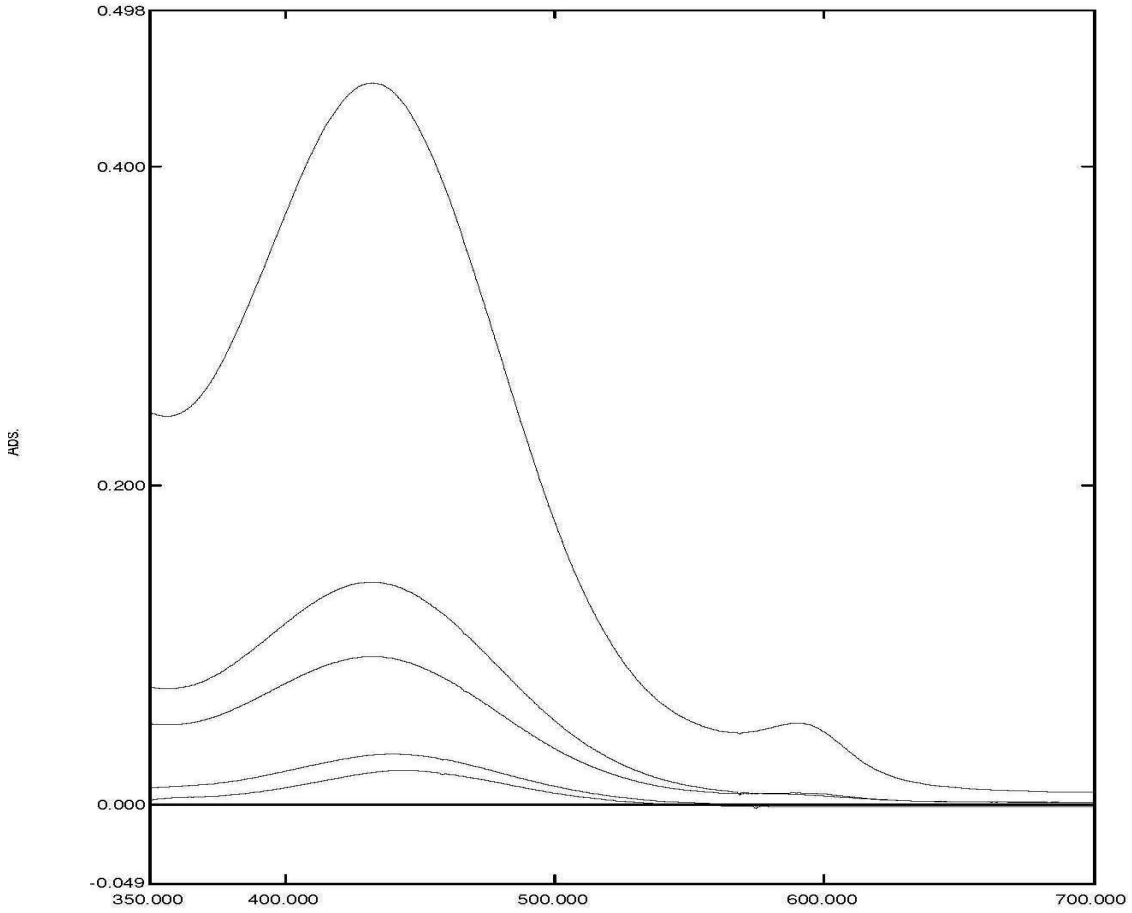


Table 19: Results of Assay and Precision of VAL

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	40mg	40.04 ± 0.0003	0.0083	100.01 ± 0.8382	0.0083
2.		40.38 ± 0.00034	0.0086	100.95 ± 0.8724	0.0086
3.		40.45 ± 0.00033	0.0082	101.12 ± 0.8382	0.0082

**Each value is the mean of three determinations*

Recovery studies

To study the accuracy, precision and reproducibility of the proposed method, recovery study was carried out by adding a known quantity of drug to preanalysed sample and the percentage recovery was calculated and the results obtained are presented in

Table 20: Results of Recovery Studies of VAL

Expected % Recovery	Amount of Drug Added in mg		Total Amount Assayed (mg) *	Amount Rcovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	40mg	8	48.53	8.08	20.19	100.96 ± 0.241	0.0119
50%		20	60.38	19.93	49.83	99.66±0.3988	0.0080
100%		40	80.25	39.80	99.50	99.50 ± 0.4605	0.0046

*****Each value is the mean of three determinations*

PRACTICAL ASPECTS OF Q-NMR

High selectivity under appropriate acquisition condition with any available pure standard compound containing the nucleus of interest has made qNMR a popular method of analysis. There are a number of issues and parameter to be considered when measuring NMR spectra for quantitative analysis. Some of the parameters which ensures accurate and precise quantitative analysis are briefly given below:

Internal Reference Standard (I.R.S).

The I.R.S chosen should be available in a pure state which can be structurally unrelated to the analyte but should contain the nucleus of interest and has a resonance that does not overlap with that of analyte. The other requirements for standards being, they are chemically inert, have low volatility, have similar solubility characters as the analyte and should have reasonable relaxation time. Based on these conditions. Tetra chloro nitro benzene (TCNB) which had the said characteristic was chosen as internal reference standard for our quantification.

Internal standard and concentration of analyte

In qNMR spectrum the integrated intensity of a resonance due to analyte nuclei is directly proportional to its molar concentration and to the number of nuclei that give rise to that resonance.

$$\frac{Integral\ area}{No.of\ nuceli} \propto concentration$$

On comparing the integral of an analyte resonance to that of standard compound of known concentration, the analyte concentration can be determined.

Normalized area std

$$Analyte\ concn. = \frac{Normalized\ area\ of\ analyte}{Normalized\ area\ of\ std} \times Std\ conc$$

The direct proportionality of the analytical response and molar concentration is a major advantage of NMR over other quantitative spectroscopy. (The Quantitative NMR Portal, http://tigger.uic.edu/~gfp/qnmr/)

Choice of Nuclei

A wide range of nuclei can be measured, with the spin ½ nuclei ^{1} H, ^{13}C,^{15}N, ^{19}F, ^{29}Si and ^{31}P above among the most common. Most qNMR experiments make use of ^{1}H because of inherent sensitivity of this nucleus and its high relative abundance (nearly 100%). Compared with other nuclei like ^{13}C, ^{1}H nuclei have more favorable T_1 relaxation times.The choice of nuclei also depends on whether one nucleus universal detection (for organic compounds ^{1}H, ^{13}C, fall into this category) or selective detection (e.g fluoride ions in ppm level in water)

Sample concentration

In NMR the signal detected scales linearly with concentration. Since qNMR is not a very sensitive method, reasonably concentrated samples are used, for protons this means analyte concentration will be in millimolar to molar range, depending upon the instrument. Other nuclei are less sensitive than proton (^{1}H 100% abundance) (G.F.Pauli, 2007)

ACQUISITION PARAMETERS

Homogeneity of magnetic field

The field homogeneity is adjusted through a process known as shimming. The NMR samples should be free of particulate matter because particles, hinder shimming, accurate shimming is important for integration.

Number of Scans

It is important to generate spectra that have a high signal-to-noise ratio to improve the precision of the determination. The signal to noise ratio should be higher than 1000:1 for integration errors of less than 1% .This is achieved easily in spectrometers with indirect probes. (Engineer -2010)

Acquisition Time

The acquisition time (AT) is the time after the pulse for which the signal is detected. Because the FID is a decaying signal, there is not much point in acquiring the FID for longer than 3 X T_2 because at the point 95% of the signal will have decayed away into noise. Typical acquisition times in ^{1}H NMR experiments are 1-5 sec.

Repetition Time

The repetition time is the total time between the start of acquisition of the first FID and the start of acquisition of the second FID. The repetition time is the sum of the acquisition time and any additional relaxation delay inserted prior to the rf pulse.

Pulse Width

The NMR signal is detected as a result of a radio frequency (rf) pulse that excites the nuclei in the sample. The pulse width is a calibrated parameter for each instrument and sample that is typically expressed in µs.

Integration regions

The shape of an NMR peak is given by the Lorentzian function $L(x)= h/[1+(x/w)^2]$, where h is the peak's height and w is its line width at half height. Obviously, we can't integrate every peak from -infinite to +infinite, so we must set a practical limit. Griffiths and Irving have shown that in order to cover 99.0% of the area of any peak, the region must spread over a range that is at least 25 times the line width of the peak in both directions.

Baseline correction

NMR integrals are calculated by summation of the intensities of the data points within the defined integration region. Therefore, a flat spectral baseline with near zero intensity is required.

Materials and Methods

All the chemicals used throughout the experiment are of highest purity analytical grade.

Solvent used: Deuterated chloroform ($CDCl_3$) was procured from Merck India Limited.

Internal standard: tetra chloro nitro benzene obtained from Merck India Limited.

Bulk material: Authentic sample of valsartan was generously gifted from Madras Pharmaceutical Limited.

Dosage form: valsartan was purchased from local market.

Instrumentation

All ^{1}H NMR spectra were acquired using JEOL AL 300 FT-NMR spectrometer operating at 300.13 MHz. 32 free induction decays (FIDs) were collected for each sample into 32,768 data points using a spectral width of 6,001.50Hz; digital resolution of 32768/6001.5=5.459points /Hz and an acquisition of time of 5.46 seconds. The chemical shifts were referenced internally to tetra methyl silane (TMS, δ =0.0), The spectrometer is equipped with 5 mm Z- gradient BB probe using 1H 90° pulse width of about 11 µs. Relaxation delay of 60s was applied during the analysis. The ^{1}H NMR spectra were processed and handled using AL 300 software.

Method

Calibration of the standard

An aliquot weighed quantity of the internal standard TCNB was dissolved in $CDCl_3$ to obtain the concentration of 30mg/mL. The standard drug VSN was weighed and dissolved in the above prepared internal standard stock solution to obtain a concentration of 150.05mg/mL. The standard stock solution was further diluted to obtain 12, 13.5, 15, 16.5 and 18mg/mL of VSN. The NMR spectra were recorded for each solution and calibration chart was plotted between integral value along Y-axis and concentration along X-axis. Two integral values one at 1.73ppm and the other at 4.9ppm were considered.

Fig14: NMR spectra of standard valsartan

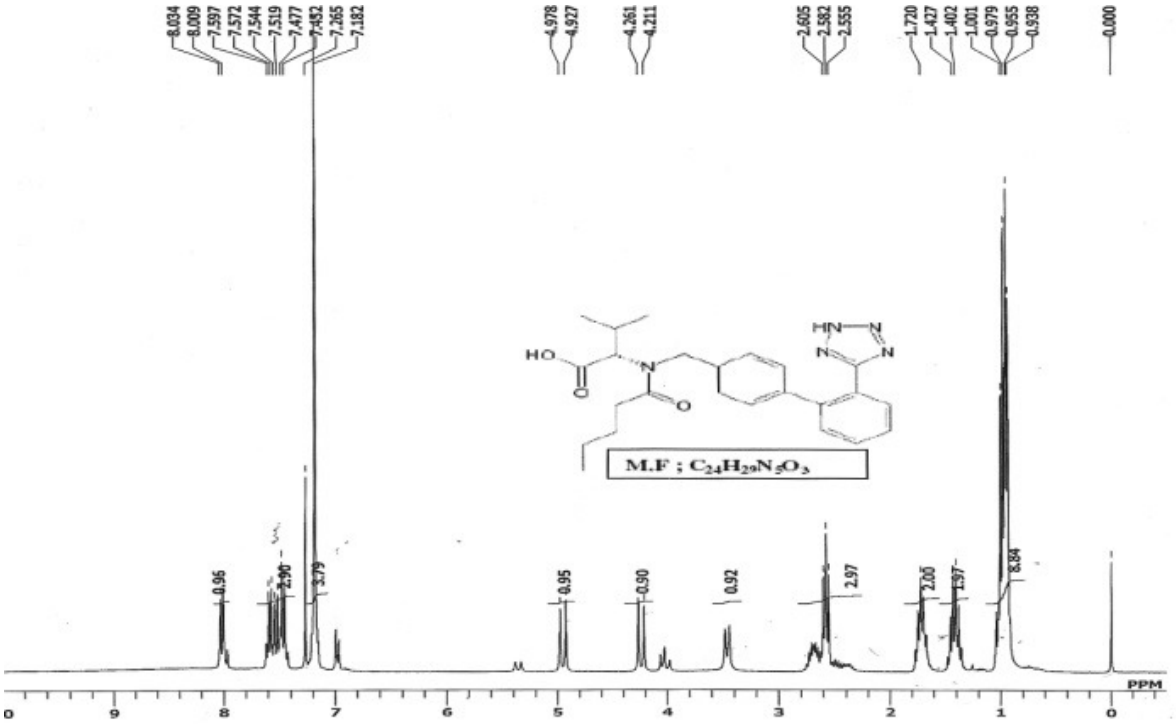


Fig15:Comparative NMR spectra of standard and sample valsartan

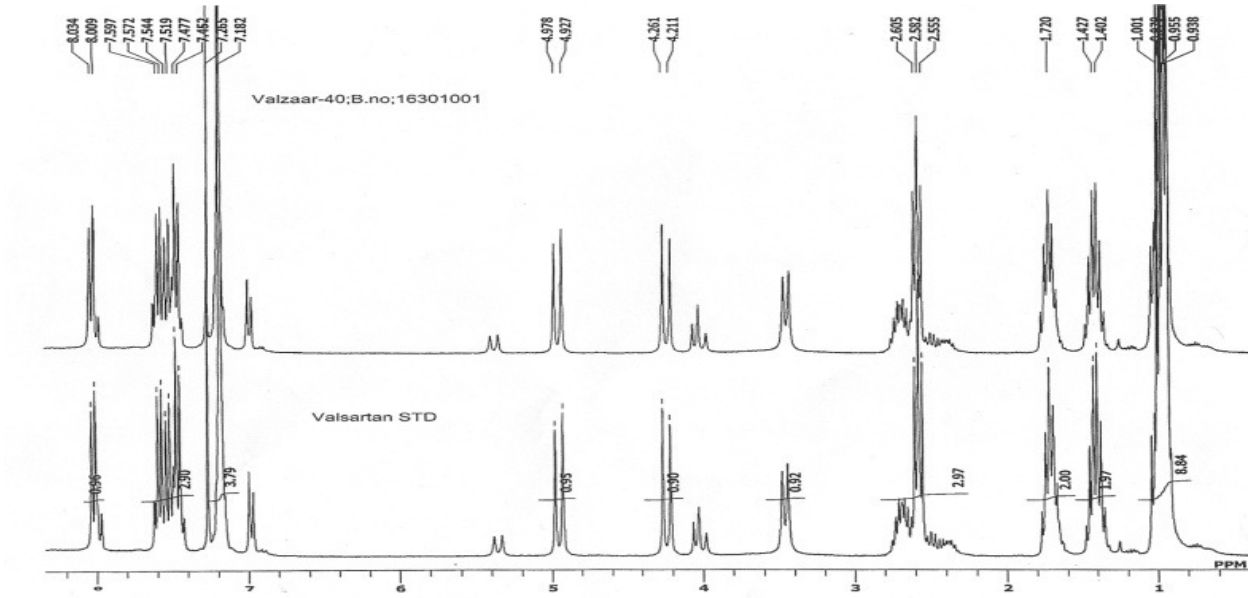


Fig16 NMR spectra of sample valsartan with internal standard (TCNB)

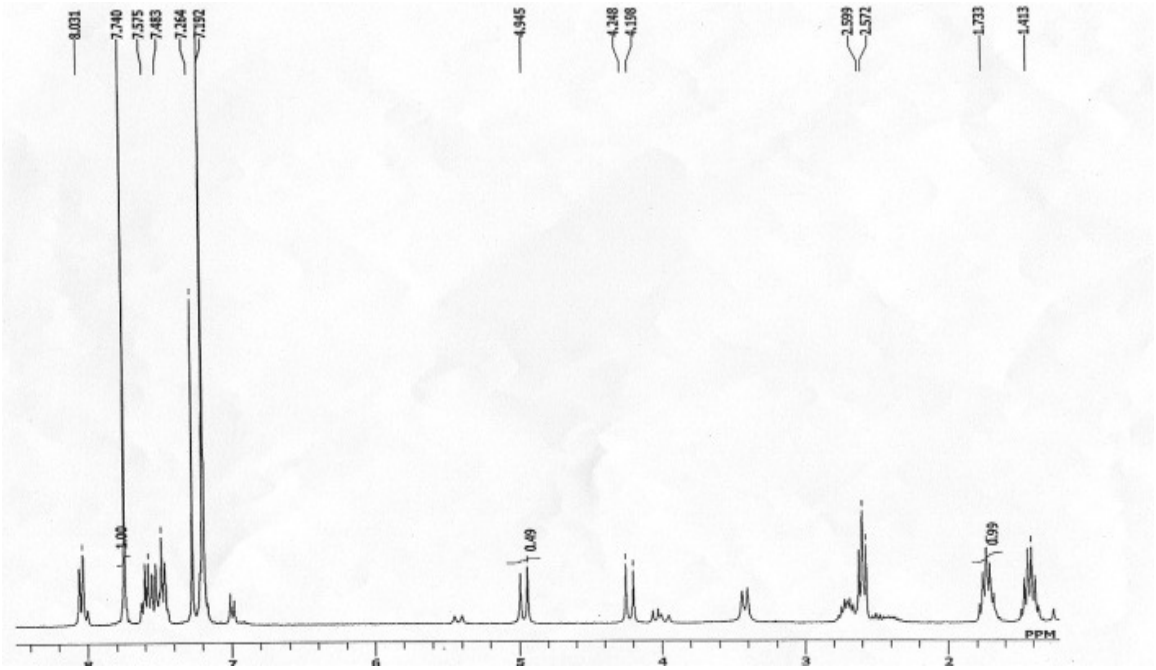


Fig17: Calibration chart of Valsartan at 1.73 ppm

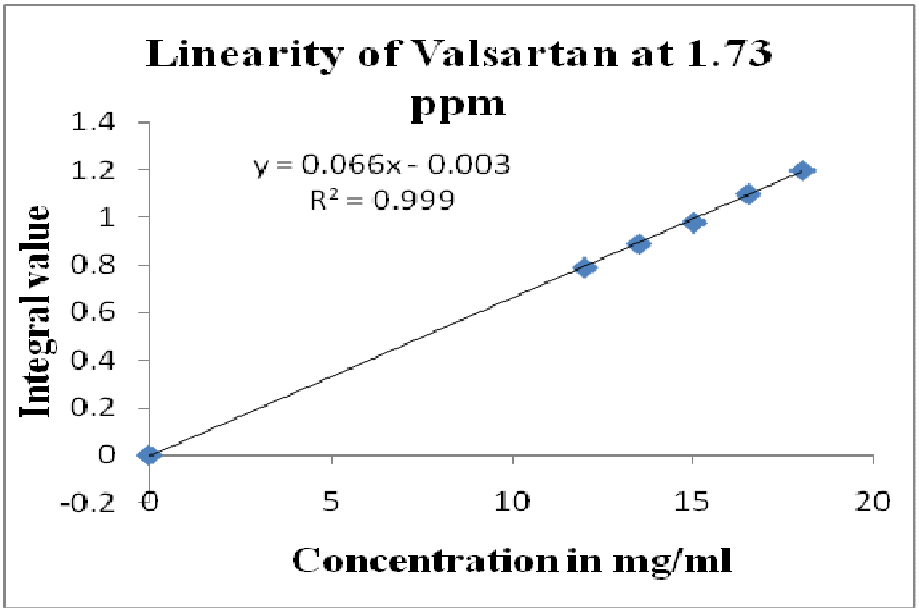
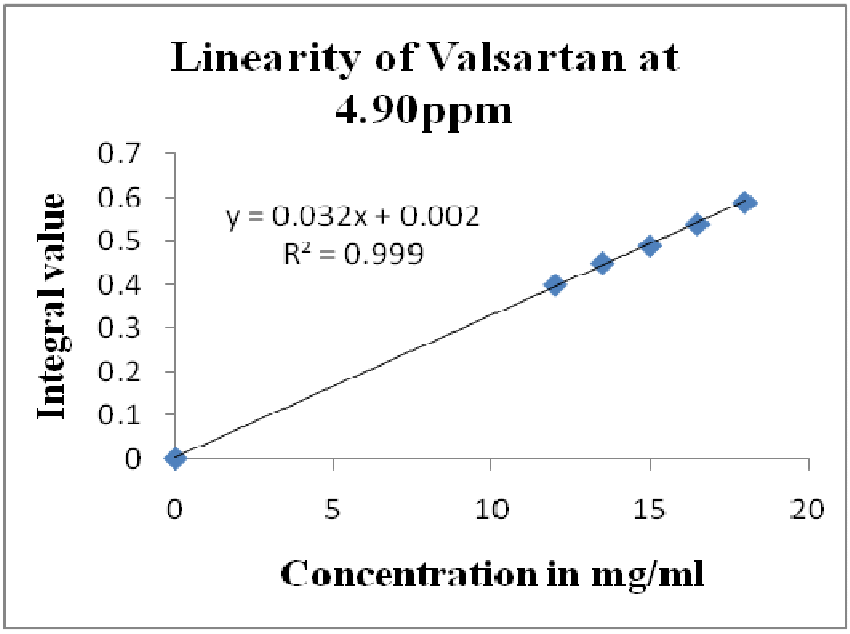


Fig 18:Calibration chart of Valsartan at 4.90 ppm



Assay

Weighed 20 tablets of VSN and ground to fine powder. Accurately weighed tablet powder equivalent to 15mg of VSN and transferred to a 1mL standard flask. Weighed accurately 15mg of TCNB and transferred to the same standard flask. Added 1mL of $CDCl_3$ and warmed gently for 5 minutes, the mixture was sonicated for 2minutes and filtered through Whatmann filter paper No.41. About 0.6mL of the solution was transferred into a 5mm NMR tube. The NMR patterns were then recorded under the given experimental condition and the signals corresponding to the chemical shift of 1.73, 4.95, 7.74 ppm were integrated and further used for quantification. Six replicate analysis were performed to assess the precision. The concentration of the analyte in the sample can be calculated using the given formula.

$$Ss = \frac{Is \times Wr \times Ms \times Nr \times Sr}{Ir \times Ws \times Mr \times Ns \times Ss}$$

Where, Is and Ir are the integral value of the sample (VSN) and internal stanadard, Ws and Wr are the weight taken, Ms and Mr are the molecular mass and Ns and Nr are the number of protons under consideration for sample (VSN) and the internal standard respectively. Table:21

Table21 : Results of assay of Valsartan

Drug	Label claim	Amount present ± SD	RSD	% Purity* ± SD	RSD
Valsartan	40 mg	39.19 ± 0.256	0.652	97.97±0.586	0.598

Each value is the mean of six determinations

Accuracy and precision:

The precision of the developed method was assessed by performing the assay of the formulation for about six times. Accuracy of the method was ascertained by the standard addition technique (recovery). Known amount of the standard VSN was added to the previously analysed sample. This was performed on three spiked levels and the NMR patterns were recorded. Six replicate analysis of above said procedure was performed.Table:22

Sample	Assay (Content of Valsartan on mg/tab)
Precision-1	39.06
Precision-2	39.36
Precision-3	39.45
Precision-4	39.44
Precision-5	38.97
Precision-6	38.87
Mean SD % RSD	39.19 0.256 0.652

Table 22: Recovery studies

Amount of drug added		Recovery using integral value at 1.73 ppm			Recovery using integral value at 4.9 ppm		
Tablet powder added	Standard (VSN) mg/mL	Amount Recovered mg/mL	% Recovery ± S.D	RSD	Amount Recovered* mg/mL	% Recovery ± S.D	RSD
15 mg/ml	12	12.05	100.35 ± 1.44	1.44	12.05	100.38 ± 1.55	1.15
	15.01	15.01z	100.08 ± 0.74	0.73	15.07	100.43 ± 0.75	0.75
	18.01	18.04	100.23 ± 1.36	1.36	18.05	100.28 ± 1.83	1.83

Each value is the mean of three determinations

RESULTS AND DISCUSSION

Assignment of ^{1}HNMR Signals of VSN

Structure formula of Valsartan is shown in the introduction part of this article. The multiplets from 0.938 to 1.001δ is attributed to the aliphatic (-CH_3) protons. The signals at 1.7δ can be attributed to the H_4 (-CH_2) protons. The signal at 1.402 can be attributed to H_5 (- CH2) protons. The singlets at 8.0δ could assigned to the H_3 (-NH) proton of the tetrazole ring as they resonate downfield. The signals at 7.452δ and 7.519δ can be assigned to the aromatic protons H_1 and H_2. The signal at 2.5δ could be assigned to the H_6 (-CH_2) protons. The signals at 4.2 δ and 4.9 δ are due to the resonance of the (-CH) protons. The –OH proton of the carboxylic acid group is off the chart to the left which is a characteristic of carboxyl hydrogen. The NMR spectrum of the blank i.e., TCNB in $CDCl_3$, represented only a sharp single signal at 7.74δ due to the proton of TCNB at its 4th position.

Characteristics of the internal standard

2, 3, 5, 6- tetra chloro nitro benzene was used as the internal standard. TCNB was selected as the internal standard as it is chemically inert and is less volatile. The NMR studies of TCNB showed that it had a good acceptable relaxation time T_1 and produces a sharp single signal due to single proton at the 4th position at a chemical shift of 7.74 ppm which does not overlap with the signals of the analyte VSN. TCNB was soluble in $CDCl_3$ same as that of the analyte VSN. Moreover TCNB is available in pure form and is a crystalline solid at room temperature and can be dried to remove water of hydration.

SUMMARY AND CONCLUSION

The present work entitled "Quantification of Valsartan by UV-Visible and NMR spectroscopy comprises of UV-Visible spectrophotometric method and QNMR methods for estimation of Valsartan. The Ultraviolet method involves the determination of Valsartan by different methods like standard absorbance method, area under curve, derivative spectroscopy and Q-absorbance method. The drug obeyed Beer's law at the concentration of 5-30µg/mL. The correlation coefficient was found to be 0.99 for all the method. The low percentage RSD value shows that the methods developed are not affected by the presence of sample matrix (or) devoid of interference by the excipients.

The visible method involve Acid dye complexation with Bromo thymol blue and Bromo phenol blue. Both the methods obeyed Beer's law at the concentration of 100-600µg/mL for Bromo thymol blue and 40-240µg/mL for Bromo phenol blue. The correlation coefficients were within the limit, and RSD percentage was low. Thus the methods were precise, specific and accurate.

The qNMR method of Quantification is a novel and sophisticated technique. TCNB has been used as the internal standard and the solvent used is $CDCL_3$. The intergrated intensity of resonance of valsartan at 1.73 ppm and 4.9ppm were considered for the Quantification. The standard and the sample resonance were compared and the amount present was calculated. The precision studies and the recovery studies were performed. This method was precise and accurate though makes use of sophisticated and costlier instrument.

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